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THE PROCESS OF FERTILIZATION IN ASPIDIUM AND ADIANTUM.

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INTRODUCTION.

The investigations of Ikeno, Hirase, Webber, and Shaw have given increased interest and importance to all facts bearing on the process of fertilization in plants. This interest centers on those groups which mark the boundary line between the lower and the higher forms. The correspondence between the results of Ikeno's work on *Cycas revoluta* and those of Shaw on *Onoclea* makes necessary a close study of the details of the fusion of the egg and spermatozoid for related forms. Previous investigations in both animals and plants have shown the spermatozoid as losing its distinct character and assuming the form and appearance of a resting nucleus of equal or nearly equal size with the nucleus of the egg before their final fusion. Ikeno and Shaw, on the contrary, have described the actual entrance of an unchanged spermatozoid nucleus into the nucleus of the egg which is found in the usual resting stage.

The work was begun in the Botanical Laboratory of Lake Forest University under the advice and direction of Dr. R. A. Harper, now of the University of Wisconsin. To him are due the methods of work and much help and advice in the completion of it. The actual study of the material and all of the work on the paper have been done in the Botanical Laboratory of the University of Missouri during the college years '97–'98 and '98–'99. To Dr. Ayers of the University of Missouri I owe the daily assistance and inspiration which has enabled me

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to finish the work since coming here. I am indebted also to Dr. Trelease of St. Louis for material and the use of his excellent library.

Material and Methods.

Fern prothallia may be found very abundantly in any greenhouse where ferns are kept. Most of my material was collected in the greenhouses of Lake Forest and belonged to the genera *Adiantum* and *Aspidium*. Young ferns were grown from part of the material collected and found to be species belonging to these two genera. Since working in Missouri State University I have twice succeeded in collecting material in the field under natural conditions. In both cases the prothallia grew at the mouth of caves and belonged to the species *Woodsia obtusa*. I have also obtained prothallia of the same species from the Missouri Botanical Garden at St. Louis through the kindness of Professor Trelease. I have kept such material in the laboratory under a bell jar where fertilization was accomplished and young ferns were rapidly developed.

To get stages of fertilization in these forms I have found that putting a considerable number of prothallia, removed from the soil as carefully as possible without injury, into a watch-glass and filling it half full of water, gives the best results. They are left in water from an hour and a half to three hours. The shorter time has given the more satisfactory results. The longer times have given figures which seem to be questionable. The archegonia seem to be overstimulated so that cases apparently occur where they open before fully ready for fertilization. Antheridia also seem to burst before the spermatozoids are fully ripe, so that stages where either condition occurs are at least questionable. Killing and fixing has been best accomplished in Flemming's weaker chrom-osmium-acetic mixture allowed to act for thirty to forty-eight hours. The material was then dehydrated and imbedded in paraffin. Shrinkages sometimes occurred, but my figures show that in my best preparations they were extremely slight and in many cases not to be detected at all. The sections were cut five microns in thickness and stained with the Flemming triple-stain safranin, gentian violet, and orange

G, cleared with oil of cloves and mounted in balsam. In my later studies on antheridia, I have fixed whole prothallia by exposure to the fumes of osmic acid. The antheridia were then dissected out with needles under a lens and stained with Haidenhain's iron alum-haematoxylin and mounted in glycerine. The spermatogenous cells could then be easily crushed out. This method, however, gave no better results than the study of antheridia in the sections prepared by the former method, but as a check upon the earlier work the substantial agreement between results was of considerable value.

Study and drawing have been done with a Leitz $\frac{1}{12}$ oil immersion, with a Nachet $\frac{1}{12}$ oil immersion and latterly with the Zeiss Apochromatic 3.00 mm. apert. 1.30, using the Zeiss No. 12 compensation ocular and the new Bausch & Lomb camera lucida — giving a magnification of 1700 diameters.

General Descriptive Notes.

A description of the general morphology of the archegonium and antheridium in the group would be superfluous. A few observations upon the special distribution of organs on prothallia may be of value. Prothallia bearing antheridia only were often found in both forms, but such prothallia rarely showed that typical heart-shape which is universally described for this group of ferns. Their outline was usually irregular, rarely showing the notched apex at all. In ordinary monoecious forms antheridia are developed much earlier than archegonia, and are freely distributed over about one-third of the surface. This area extends from the base of the notch backward. Antheridia are rarely found farther forward than the base of the notch or closer to the edge than one-third of the diameter of the prothallium. I have only once observed an antheridium developed upon the upper surface. Neither antheridia nor archegonia depart from the well-known descriptions in general structure or development. The archegonia in these forms are distributed only on a comparatively small space ranging directly backward from the notch. On older prothallia, if no embryo is developed, the area devoted to the development of archegonia becomes sometimes quite large, so that often a considerable number become ripe at once. In

one case I found spermatozoids attracted toward and actually entering six archegonia. There is usually but one or at most two fully developed archegonia at any one time except in these older and larger forms. I have sectioned some hundreds of prothallia and found but one case where an archegonium was developed on the upper side. The case occurred where the prothallium producing it was overlaid by another so that its presence was due to the conditions of moisture and nourishment induced by the second prothallium, agreeing with the result of experiment where such archegonia were artificially induced by Heinricher.

If, however, we turn to *Woodsia obtusa* and consider the prothallium in that fern for comparison, we do not find the regularity in shape and form so distinctive of the others. The prothallia here tend to grow in length with approximately a regular width. The sides are sinuate so that such prothallia do not usually lie flat upon the substratum. Here as in the others the margin of the prothallium is but one cell-layer in thickness, but instead of a gradual thickening we find a definite thickened ridge developed on the under side upon which antheridia and archegonia are produced. The production of sex organs begins somewhat earlier in this form, and continues in the newer portion of the gradually lengthening prothallium until a fertilization is accomplished.

In contrast to the observations of Jeffrey on *Botrychium*, I found but one case among the large number examined where two archegonia upon the same prothallium had been fertilized, and produced embryos. In this case (fig. 49) the two embryos formed lie so close together as to indicate a possibility of the ultimate fusion of the two had their development been continued. This observation is of interest on account of its bearing upon the views of polyspermy in ferns, advanced by Lowe ('95) in his "Fern Growing," where he finds on different portions of the same plant the characters of as many as three or even *six* (!) different species. He has not attempted to determine by histological methods whether this remarkable phenomenon results from the fertilization of three or more separate oospheres by spermatozoids of as many different species, and a later fusion of the embryos into a sort

of "*composite plant*," or a true case of polyspermy. The presence of a number of spermatozoids in the neck of the archegonium or even in or upon the egg itself is common, and has been many times described. But there does not seem to be any positive evidence that more than one spermatozoid actually unites with the egg — or should such cases occur, that the embryos so formed would develop into ferns. Lowe accepts the theory of polyspermy, but bearing in mind that the fertilized egg is a single *cell*, and as such should act as a unit, the resulting hybrid, if it reached maturity at all, would be expected to show uniform characters. It is, however, entirely possible that two such embryos as shown in figure 49 could fuse to produce a single stem so that their individuality would be, apparently at least, lost. In fact the very closeness of the archegonia to each other would make such a result easily possible and rather difficult to avoid without the crowding and dwarfing of both or the actual destruction of one.

The Spermatozoid.

Before attempting to discuss the process of fertilization we must first consider the gametes separately. Belajeff ('97) gives the following as general for the *Filicineae:* "Das reife völlig entwickelte Spermatozoid besteht in seinem hinteren Theile aus einem ziemlich dicken spiralformigen Chromatinkörper der von einer dünnen Plasmahülle umgeben ist." — "Das vordere Ende des Spermatozoidenkörpers hat eine bandartige Form, reagirt wie Plasma und scheint gewissermassen eine Fortsetzung der Plasmahülle des Kernes darzustellen." He also notes that it carries with it a cytoplasmic vesicle or remnant at its posterior end. In my preparations the ripe spermatozoid consists (figs. 4, 4a) of a large nuclear portion tapering from a blunt end behind to a slender but not sharp point forwards and thrown into from two to three turns of a spiral. The number of turns depends to a large extent upon whether the spermatozoid is in motion or at rest, free or crowded together with others into the neck of the archegonium. At the pointed end of the nuclear portion I could determine by careful staining about one and a half short turns of the spiral which contain no chromatin and must be regarded

as cytoplasm. At the end of this cytoplasmic portion an enlargement may sometimes be demonstrated (fig. 4) at or near which the cilia seem to be attached. I have not been able to stain them to show their actual points of attachment. At the time of fertilization I have not been able to distinguish any cytoplasmic vesicle attached to the blunt end of the nucleus, although Belajeff describes it as present. Shaw describes it as actually torn from the moving spermatozoid as it becomes entangled in the slime at the mouth of the archegonium. I have observed it in the free swimming spermatozoid. The nuclear portion is enveloped by a cytoplasmic covering (Hülle of Belajeff) which appears to be continuous with the forward coils which bear the cilia. This envelope or covering has been variously described. Guignard claimed that the entire body of the spermatozoid except the cilia and the vesicle behind was nuclear. Of this the forward coil bearing the cilia showed no chromatin though it was continuous with the chromatin-bearing portion. Belajeff finds the anterior end a cytoplasmic band inclosing a deeply staining fiber or thread derived from the "Nebenkern," curving around the nuclear portion and, presumably, inclosed in the same cytoplasmic covering (Hülle) as the nucleus. Schottländer described in addition to the same cytoplasmic envelope a band or "Segel" the whole length of the nuclear portion. I have seen a similar appearance at times, but on the basis of my observations of the living spermatozoid and the fate of its several parts in the fertilization process, I am convinced that the cytoplasmic envelope does not fit the nucleus closely. The nucleus lies in one side, so that the appearance of a band may be due to the collapse or partial collapse of the portion not distended by the nucleus. This explanation would correspond more nearly with the observation of Shaw for *Marsilia* where he finds a thread derived from the "blepharoplast" not only in the forward coils but traversing the full length of the nuclear portion and even extending out behind it. It also agrees with the statement of Belajeff that the thread derived from the "Nebenkern" lies in one edge of the cytoplasmic band which constitutes the forward end, while the pointed end of the nucleus

appears on the opposite edge of the band in the third turn of the spiral.

In considering the function of the spermatozoid and its various parts, it is necessary to go back and review to some extent at least its development. The antheridium contains a number of "spermatozoid mother cells" ("spermatogenous cells" or spermatids, if we are to use the same term as the zoologist), varying from 32 to probably 128. The prothallia differ in the number produced, and often antheridia on the same prothallium seem to contain different numbers. The number probably varies inside these limits, however. Belajeff observes that the spermatogenous cell contains a large nucleus which comes to lie upon one side of the cell (fig. 28) close under the wall. At this time, according to his statement which he makes general for the *Filicineae*, there appears in the cytoplasm a round granule or body staining more deeply than ordinary cytoplasm and sharply marked off from it. He describes this body under the name of "Nebenkern." Shaw observes and describes the same body under the name of "blepharoplast" and finds it present in the mother cells of the spermatids in *Marsilia* and *Onoclea*. That is, according to Shaw, the blepharoplast in these forms must divide with the division of the cells at least once before it assumes its final form. This is unquestionably the same body which was described by Webber for *Zamia* as "blepharoplast" and has since been decribed by Ikeno for *Cycas* as a centrosome. In my material, at a stage just preceding this, the nucleus contains a network bearing large chromatin granules which are disposed, for the most part at least, close under the nuclear membrane. There are from one to three large nucleoli which stain a clear red with safranin while the chromatin takes a deep blue from the gentian violet (fig. 27). Comparing this figure, where changes have already begun, with earlier stages, there seems to be a rapid increase in nucleolar material. In many cases the nucleoli appear to have just divided. All of the cells of the antheridium appear to be in nearly the same stage, and there is a great regularity in the successive changes as they occur in cells of the same group. This regu-

larity is so marked and so extensive at times that all the spermatids of an antheridium are not only in the same stage of development at one time, but often appear to develop spermatozoids which are oriented in the same way in the cell.

I find the so-called "blepharoplast," or "Nebenkern," as a round body lying outside the nucleus toward the center of the cell, usually. It stains as the nucleoli within the nuclei did before its appearance. Sometimes it is connected by strands or fibers with the nucleus — more often such connection could not be demonstrated. The body, however, seems to be present in all cases. It is noticeable that the nucleoli, as distinct bodies, disappear *before* the appearance of this body. At least I have not observed both as present at the same time. This disappearance of the nucleoli at the time of the appearance of the blepharoplast would suggest a relationship between them, in material at least. They stain alike. The blepharoplast when first seen occupies a position which, to say the least, could easily have been occupied by one of the nucleoli before the nucleus left its position at the center of the cell for the secondary position near the wall. Further, the nuclear membrane, if it remains intact, has become so thin that it is difficult to follow. Although I have not observed its origin from the nucleoli, such a theory goes farthest toward explaining the changes which occur. Such a theory, coupled with the fact that it contains kinoplasm, as shown by its function as a bearer of cilia, would agree with the conclusions of Strasburger ('97) as to the kinoplasmic nature of the nucleolus.

I cannot confirm the observations of Belajeff, Ikeno, and Shaw, that the first step in the development of the spermatozoid is the stretching (Ausdehnung) of this body first into a crescent, then gradually into a fiber which forms a dark line on the edge of the cytoplasmic anterior end of the spermatozoid. I have observed it many times unchanged when the nuclear portion of the spermatozoid had developed into a whole spiral turn, and sometimes even more. Many of the later stages of spermatogenesis and adult figures, as figure 4, indicate that this body comes to lie at

the tip of the cytoplasmic end of the spermatozoid. The nature of the body is still questionable. It has been shown by Belajeff for the *Equisetaceae* to be the origin of the cilia. Webber, Ikeno and Shaw find the same to be true. This function points toward a relationship with the centrosome in lower plants as described by Strasburger or in animals as has many times been shown. But beyond serving as the origin or point of attachment of the cilia it develops no function. It does not act as a centrosome in nuclear division (see Ikeno's own figures), and is lost before fertilization, so fails to perform the two chief functions of a centrosome. Guignard, investigating the spermatogenesis of *Chara*, found the earliest change was the formation of a darkly staining line just inside (possibly outside) the nucleus which develops into the head end of the spermatozoid and contains little or no chromatin. His description, although he regarded the body as inside the nucleus, corresponds to a large extent with the recent observations of the development of the blepharoplast. In my own material no such change appears at first. I have not been able to determine exactly when the cytoplasmic forward or head end is formed, but certainly the earliest change involves only the nucleus.

Our chief interest, however, lies in the transformations which occur in the nucleus. In figure 27 we see the notable changes taking place. The nuclei have become elliptical and in some cases pointed at one end. The chromatin appears in the form of granules deeply stained (blue) on a network of achromatic material or linin. This network is massed near the surface of the nucleus. The prominent nucleoli (stained red) occupy a more central position. Two of them appear in each cell, but in figure 28 part of the cells contain three instead of two, so that although the position of the nucleoli with reference to each other indicates multiplication by division, the number present is not essential. The nucleus now lying very near one side of the cell becomes flattened tangentially into a band lying just under the wall with its concave depression toward the center of the cell. In this concavity we find the blepharoplast (figs. 29, 36). The flattening process is accompanied by the

elongation of the nuclear body into a pointed and a blunt end (figs. 29–37), with an enlargement at its center. This development into a pointed end may be abrupt as in figure 31, where it is seen from above, or appear as gradual (figs. 27, 37, 38). Of these two ends the more pointed is usually the longer. The chromatin material is now seen in short thick masses or bars lying on the surface; the achromatic material cannot be distinguished clearly. The bars of chromatin begin to connect together into a network. The nucleoli are no longer to be seen, but there appears to be a red-staining ground-work or central mass to the whole nucleus. The bars of blue-staining chromatin stand out sharply on the surface of this red-staining central mass. The further changes in the nucleus are a thickening and connecting together of these chromatin bars, the lengthening and continued flattening of the body. In figure 41 the anterior end of the nucleus appears to taper gradually towards a blunt point; the posterior end is spread out into strands. In this figure the cytoplasmic anterior end has become nearly one-fourth of the turn while the nuclear portion has reached about one and a half spiral turns. The chromatin is connected together into strands with a tendency to longitudinal arrangement, with crossing bars of the same material. Within this network of chromatin there is a distinctly red-staining mass. Similar proportions have been developed in figure 40.

As the spermatozoid ripens, the chromatin mass is transformed into a dense layer over the surface of the now band-like nucleus, which explains Belajeff's observation that it is a homogeneous chromatin mass. If, however, we examine the cross-section, as he does not appear to have done, we get further evidence. In figure 42 we have a drawing of an actual cross-section of a spermatozoid lying in an archegonium upon an egg-cell magnified 2550 diameters. The chromatin here appears as a deep blue layer on the outside of a red central body. The nuclear body here is seen to be flattened, concave on the inner side of the coil, convex on the outer side, and perhaps a little thicker at the edges than in the center. The nuclear portion of the spermatozoid is about forty microns in length, perhaps three microns in greatest

diameter. If it were a solid mass of chromatin, it would require much more such material than we find in the nucleus of the spermatid, which is scarcely six microns in diameter. Schottländer in his observations on the spermatozoid of *Aneura pinguis* finds a similar ground substance in the center of the nuclear portion surrounded by a chromatin layer. Zacharias ('87) in investigating the spermatozoids of *Chara* has shown by micro-chemical means that the ciliate anterior end and vesicle behind are cytoplasmic. By digestion experiments he succeeded in dissolving the chromatin, leaving the anterior end and the cytoplasmic posterior end connected by the cytoplasmic envelope and a delicate fiber (zarte Faden) in the center whose nature remained undetermined. By digesting the nuclear portion Strasburger demonstrated the cytoplasmic envelope of the nucleus but does not mention a central filament. Belajeff by a similar experiment found the two ends connected by a slender thread, which may have been either this central filament or the cytoplasmic layer on the outside. These tests show that the cytoplasmic forward end and the connecting fiber react in the same way as the kinoplasm of the spindle fibers, which would fix them as kinoplasmic structures. The same central filament is described by Franze ('93) as "Achsenfaden." On the basis of my own observations it seems to be nuclear in origin and probably represents some transformation of the large amount of nucleolar material present in the earlier stages.

To recapitulate the structure of the spermatozoid of the fern as now understood: — The entire body forms a left-handed screw or spiral about forty microns in length and possibly three microns in greatest diameter. The anterior cytoplasmic portion forms about two spiral turns inclosing a body or thread derived from the "Nebenkern" or "blepharoplast" which bears the cilia. The central portion of the two much larger and longer coils consists of the nucleus and its cytoplasmic envelope. The diameter of the nucleus increases from a pointed end in front to a blunt end behind. The posterior portion is a cytoplasmic vesicle attached to the inside of the last coil of the nucleus. The cilia, according to Belajeff, exceed forty in number. They are, for the most

part, at least, directed backward and exceed in length the body of the spermatozoid, instead of being comparatively short and directed outward as they have previously been described and figured. Their motion passes backward in waves from their origin at the anterior end of the body toward their free ends, so that as much as two waves of motion can sometimes be seen at once following the curves of the body of the spermatozoid backward. The cytoplasmic coils and at least the first turn of the nucleus seem to be actively motile also, so that in addition to the whirling motion imparted by the cilia the spermatozoid undergoes a rapid succession of changes of form. I have not determined just how much power of movement may be attributed to the nucleus itself, but the fact that the nuclear portion of the spermatozoid enters the egg without assistance from the cytoplasm proves that it has that power in some considerable degree at least.

The Egg-Cell.

The egg-cell lies in the venter of the archegonium closely invested by the richly nourished cells lining the cavity in which it lies. It varies in diameter from about twenty-five microns to forty microns according to the direction in which it is measured. Its form in different archegonia varies from nearly spherical almost to lens-shaped. The ventral canal-cell lies in a concavity of its upper outer surface but usually not in the center and often very nearly at one side. The diagram (fig. 43) is a part of a vertical longitudinal section of a prothallium through the neck of the archegonium and the middle of the egg-cell. Here it is readily seen that the ventral canal-cell (v. c. c.) lies at the bottom of the open neck of the archegonium (N) but that this point lies at one side of the center of the oosphere or egg. The egg extends outward beyond the ventral canal-cell, so that it has come to lie in the side of the egg and to be surrounded by it except on the side turned toward the open neck. Now a section cut in a plane parallel with the line A-B and perpendicular to the plane of the section in figure 43 will explain such appearances as the open cavity *v* which appears in figures 10 and 11 and in the diagrams 46, 47 and 48, which are camera outlines

of sections of egg-cells. The opening *v* is then only the cross-section of the cavity which the ventral canal-cell occupied at an earlier stage as figure 43. In figure 44a the edge of the cavity is indicated by the dotted curved line as lying below the plane of the section; in 44b and 44c, which are succeeding sections of the same egg, it becomes very prominent. If the cell were followed through, the same appearance as 44a would be reached on the other side, or if a median section perpendicular to the plane of 44c were taken the figure would be nearly the same as 44c. Such openings surrounded by egg-cytoplasm can easily be mistaken for a "receptive spot" developed in the egg itself when they are really outside the cell. This concavity is not uniform in its development, nor when present does it always show as in the diagrams. It lies at the bottom of the neck of the archegonium. The position of the neck of the archegonium varies from almost perpendicular to the surface of the prothallium (fig. 2) when the ventral canal cell lies near the center of the egg, to an angle of less than forty-five degrees (figs. 1, 9 and 43) when the cavity usually lies far to one side. The egg-cell adapts its form to the shape of the cavity in which it lies. This concavity in its surface is not always present. In the egg shown in figures 6, 7 and 8 there was no such spot in any section. Instead we find a decided convexity of surface while the spermatozoid (*s*) is seen entering the cytoplasm a little to one side of its pointed end. This seems to be but another evidence of the amoeboid character of the egg. Shaw describes such figures as this, as a change from a concave surface to a convex surface occurring in the living cell as soon as the pressure of the ventral canal-cell is removed. He suggests that it is a readjustment due to turgidity of the egg and states that it returns to its former shape as soon as the spermatozoid has entered. Such a change from a concave surface to a convex surface probably did occur in the egg shown in figure 6, but it cannot be a constant phenomenon, for I have observed it only once or twice in the large number of archegonia examined. Further I see no evidence of any return to the former shape either in figure 6 or in any other sections examined,

so that I doubt if such a change occurs in these forms in any case.

The nucleus of the egg in its early stages shows the ordinary spherical form with one or two large nucleoli and abundant sharply stained chromatin. As the egg nears maturity, the chromatin becomes indistinct with the ordinary stain, the regular contour of the nucleus disappears, and the only prominent feature remaining is the large nucleolus. The nucleolus itself shows commonly an irregular form and appears vacuolated. Careful staining and examination enables one still to see a nuclear network upon which fine granules are distributed. These granules stain blue with gentian violet. This distinct staining reaction and the marked difference between these and the cytoplasmic granules outside justify the claim that this material is chromatin, which is only in a very finely divided state. Such networks can be seen in figures 4, 5, 6, 7 and 8, where the necessity of distinguishing chromatin from cytoplasm by a difference in shade in the drawing has made the nuclear network more prominent than it really appears in the microscope. The difficulty of showing the presence of chromatin in the unfertilized egg led Zacharias, in 1887, to say that no chromatin framework had been demonstrated in the egg of *Pteris serrulata*, *Marchantia polymorpha*, *Pinus sylvestris*, *Thujopsis dolobrata*, *Lilium candidum*, *Monotropa hypopitys*, or among animals in *Unio* or *Rana*. The same difficulty has recently been noted by Gardiner in *Polychaerus caudatus*. Chromatin is easily stainable in the last division spindle in the formation of any one of these eggs. It may be readily demonstrated in the younger stages of the egg itself, but in the ripe egg Zacharias could find no satisfactory micro-chemical evidence of its presence at the time of fertilization, although a plastic framework could be found. These observations seem to show that at the time just preceding fertilization the egg chromatin differs so greatly in its distribution at least, if there is no change in its chemical character, that it fails to give the ordinary reactions which so easily demonstrate its presence before and after this period. During the entire time the nucleoli maintain their size and position unchanged.

The whole nucleus now seems to be irregularly crescent-shaped, with its concavity parallel with the concave depression in the outside of the cell (figs. 6, 7, 8, 10, 14, 25). Careful tracing of the nuclear membrane shows it still sharp, but thin and thrown into folds like pseudopodia. The difference in staining reaction between the nuclear material and cytoplasm makes it possible to follow the delicate pseudopod-like branches of the nucleus through the cytoplasm. Such a branching condition of the nucleus rendered adequate representation of it in drawings unsatisfactory, so finally the plan adopted was to fix upon some point in the section, sometimes the upper cut surface, sometimes what I determined was the optical section, again some point which was important, and draw the outline of the nucleus in that plane. Within such figures I have represented the spermatozoids as they enter, as nearly as possible in perspective maintaining their true relation to the plane of the figure. Sometimes it was necessary to represent by a change of shade more than one plane of the egg (figs. 13, 17, 25) because a portion of the spermatozoid lay in the cytoplasm outside the nucleus but was partially overlaid by it in the plane of the figure. At its maturity, then, the egg contains a large amount of cytoplasm which appears as a granular network staining evenly for the most part, yet showing in places larger granules or microsomes whose nature has not been determined. The cytoplasmic network often shows an irregularly radial arrangement into fibers and rows of granules directed outward from the nucleus. The shape of this mass of cytoplasm is determined by the cavity in which it lies. Within it is the irregularly branching nucleus which usually occupies the center of the cell body. It approaches nearest to the surface, however, at the base of the depression which is filled in the earlier stages by the ventral canal-cell. This is the point where fertilization occurs, for here the entrance of the spermatozoid nucleus into the egg nucleus is most easily accomplished.

Fertilization.

The actual fusion of the two nuclei has been observed in only a few plants. In 1869 Strasburger observed the entrance

of the spermatozoid into the archegonium of *Pteris serrulata* and in *Ceratopteris.* He saw it disappear within the egg itself and observed the formation of a membrane around the fertilized egg within a very few minutes afterward. The entire process required a very few minutes, but the actual relations of the nuclei were scarcely touched. A fusion of the two nuclei in the resting stage has been described by Wager and Harper in fungi, by Oltmanns in *Vaucheria*, by Farmer and Williams in *Fucus.* Campbell has described changes in the spermatozoid of *Pilularia* before its fusion with the egg nucleus which point to a similar occurrence in that form. Ikeno has figured the entrance of the spermatozoid nucleus unchanged into the egg nucleus in *Cycas.* Shaw has made the same observation in *Onoclea.*

The entrance of spermatozoids into the archegonium can be easily observed under the microscope. I have not, however, attempted to control it in many cases. By removing prothallia from the soil and flooding considerable numbers in a dish together, I believe that I get the nearest approach to normal conditions. In this way I have secured a fairly representative series of stages.

After treating them as described above, the mouth of the archegonium will generally be found to contain a number of spermatozoids varying commonly from one or two to twenty or more. When the number is large they are usually so wound together that it would be hopeless to attempt to trace out the relations of each (figs. 1 and 2). Shaw has observed the spermatozoids "swimming into the archegonium, swarming over the egg and escaping again," yet he notes that as they become entangled in the slime at the mouth of the archegonium their progress is impeded and the cytoplasmic vesicle torn off. I have found that spermatozoids are attracted toward and enter archegonia which have degenerated. They may be found piled upon egg cells which have passed the stage of maturity and lost the capacity for fertilization. In such cases the amount of slime at the mouth of the archegonium would not prevent the free entrance and exit of spermatozoids. From my own observations I doubt if a spermatozoid, after once entering the

neck of an archegonium which is ripe for fertilization, escapes again, or even endeavors to escape.

Entrance into the neck of the archegonium is always effected with the pointed or cytoplasmic end forward. In entering they become usually drawn out into more, but shorter, turns. Figure 2 represents a striking condition. In this figure the two prominent spermatozoids, *a* and *b*, are directed into the archegonium *large end* first, while their pointed ends are entangled with those which lie in the outer portion of the neck. These might appear to be cases where the spermatozoids once in are endeavoring to escape. But a comparison with the spermatozoid in the neck of the archegonium in figure 4 and with figure 4a, where the spermatozoid lay just outside the mouth of the archegonium, suggests a different explanation. In figure 4a the cytoplasmic portion *c* is already disconnected and turned backward. In figure 4 the cytoplasmic end *c* of the spermatozoid *s*, though not broken off, is turned backward as if useless. A similar portion *c'* of the spermatozoid *f* seen entering the egg seems to be dragged into the cytoplasm backward. The same has also occurred apparently in *c''*, figure 6. Such figures, combined with the fertilization figures 9, 10, 11, 12, lead to the conclusion that as soon as the spermatozoid has entered the mouth of the archegonium the function of the anterior end is completed; consequently when it appears in any later stage it is merely passively dragged along. The fact that this portion is at times broken off, at times turned backward, and, as Ikeno has shown, entirely dropped before fusion, indicates that it contains nothing essential for fertilization. It contains the blepharoplast. This would prove that the blepharoplast is not essential to fertilization in the sense in which a centrosome is given that character, but a special organ differentiated for the production of cilia. In figure 2 the aggregation of the chromatin toward the ends of *a* and *b* which are inside the venter of the archegonium, compared with figures 9, 10, 11 and 12, is good evidence that each spermatozoid is endeavoring to get its entire body inside the archegonium not to escape. Combine this with the separation of the cytoplasmic end in figure 4a and its marked loss of func-

tion in *s*, figure 4, and we have sufficient grounds for saying that the attractive impulse which leads finally to fertilization must be exerted directly upon the nuclear portion of the spermatozoid. This portion, as seen in figures 2 and 4 and also in the fertilization figures 10, 11 and 12, must have the same power to respond by motion toward the egg when it lies outside that it undoubtedly has after it is inside the egg cytoplasm. In the fertilization of the animal egg (Wilson, 1896; Wheeler, 1896) the tail of the spermatozoid furnishes the motive power until the nucleus enters the cytoplasm of the egg. The tail is left outside, or if carried in is functionless. The nuclear portion often traverses many times its own diameter before fusion with the nucleus of the egg. In the fern we have a spiral nucleus in the spermatozoid, at least one turn of which is actively motile. In either case the directive influence must be exerted on the nucleus. The only difference then is that in the fern we are describing, its influence is exerted upon the nucleus alone and responded to a little earlier. The cytoplasmic portion may be dropped or become functionless as a locomotive organ before the gametes are in actual contact without interrupting or interfering with their ultimate fusion.

One or more spermatozoids finally come to lie in the depression in the upper surface of the egg (figs. 3, 4, 10) where they may be often seen coiled in the sections examined. Others may be found in almost any position upon the surface of the egg cell (figs. 1, 3). One spermatozoid now bores or forces its way into the cytoplasm of the egg (figs. 4, 6) toward the nucleus. In figure 6 there is no depression in the surface and we find the spermatozoid entering the cytoplasm near the tip of the egg. In figure 4 the depression is not clearly marked as in figure 3, but there is a marked flattening and slight concavity of surface. In both of these cases the pointed forward end penetrates first. Although not so marked in these figures as in figures 10, 11 and 12, the spermatozoid usually breaks in toward the center of the concavity of the nucleus, which usually lies closest to the surface at the base of the depression in the surface of the cell.

In figure 10 we see a portion of the spermatozoid *f* coiled in the depression in the surface of the egg which appears in

the section as a hole in the egg-cytoplasm opening to the outside beneath the plane of the section. Here a *blunt end* of the spermatozoid is seen extending through the cytoplasm, interrupting or breaking the nuclear membrane and actually entering the nucleus itself. The contour of the nucleus is drawn at the plane where the nuclear membrane is interrupted for the entrance of the spermatozoid. The appearance of this nucleus is fairly typical for a vertical longitudinal section at this stage. If the section were above the plane of this section, the nucleus would show a contour much more nearly round. We have here a figure where the nuclear portion of the spermatozoid lies partly *inside the nucleus of the egg, a part extends through the cytoplasm* and a portion still lies coiled in a depression in the surface of but *entirely outside the egg.* That is, the actual process of the union of the two gametes is before us.

In figure 11 we have a similar condition. The nucleus in this section lies very close to the surface of the cell — the bottom of the depression in its surface *v*. Only a small portion of the spermatozoid remains in this case — the larger part was carried away by the knife and will be found in the next section. Here again the portion present lies partly inside and partly outside the egg nucleus and it enters blunt end first. In figure 12 the egg cell had no depression in its upper surface. The spermatozoid has broken through the very tip of the cell. The part present shows the distinctly spiral appearance of the spermatozoid and is certainly entering large end first.

We get a more striking condition in figure 13 than in any of the others. Here one large coil and a half smaller, *a*, are seen lying outside the point *b* where the spermatozoid breaks through the nuclear membrane. The entire nuclear portion (stained deeply) lies inside the egg nucleus but is connected distinctly or rather is continuous with the coils lying outside the nucleus. In this figure the egg nucleus is drawn in the plane where the spermatozoid breaks through the nuclear membrane. The lightly colored coils of the spermatozoid contain no nuclear material. The part from *a* to *b* lies in the concave depression of the nucleus which is just below the

plane of the section, as is indicated by the dotted line *d*, which is intended to show that the cytoplasm at that point is but slightly below the plane of the section. A comparison of this with *s*, figure 4, and with figure 4a shows at once that the portion appearing now as non-nuclear, and in this case connected directly with the nuclear portion, is several times greater than the cytoplasmic forward end of the free swimming spermatozoid. But one interpretation seems to be admissible — that when the nuclear portion has forced an entrance into the egg-nucleus it breaks out of its cytoplasmic envelope and slips into the nucleus of the egg, leaving the empty envelope behind in the cytoplasm. This agrees with the statement of Ikeno for *Cycas* — "Sofort nach seinem Eindringen in die Eizelle schlüpft das Spermatozoid aus seinem Cytoplasmamantel, welcher sich bald innerhalb der Eizelle desorganisirt, während gleichzeitig der Spermakern nach dem Eikerne sich hinbewegt" (p. 595). In the fern, however, the cytoplasmic mantle, having the spiral form of the spermatozoid, can be more easily identified, to the last. It contains less cytoplasm than that of *Cycas*, consequently fits the nuclear portion more closely, so that it is carried further into the egg, while in *Cycas* it is left (Ikeno, fig. 35) at the periphery of the egg. Here it is carried as far as the nuclear membrane ordinarily, and in this one case I find it was drawn partly inside before the nucleus finally escaped. Figure 13a represents in a somewhat diagrammatic way the same nucleus as figure 13, leaving out details in shape, and turned on a vertical axis as *x*-*y* about ninety degrees. In this figure the funnel-like depression *d* lies in the upper surface a little on one side of the center. Its edge is much higher on one side than on the other. In fact this depression, to which the name "receptive spot" has often been applied, is no more regular in shape and position than the outline of the nucleus. Its occurrence is due to the fact that the nucleus conforms itself in a general way to the shape of the cell. The depression occurs in the surface of the cell, and the nucleus, having the peculiar amoeboid form, naturally shows the same depression. The spermatozoid coiled in this receptive spot broke through the nuclear membrane at the point *e* just

about the lowest point in the funnel *b*. Outside the nucleus we find a coil and a half beginning at *a* and passing through the nuclear membrane at *e*. This part contains no nuclear material and continues without chromatin through the nuclear membrane as far inside the nucleus as *c*, where it passes under a turn of the nucleus. That is, the part from *a* to *c* contains no nucleus. Passing under the coil of the nucleus at *c*, it reappears on the other side as nuclear. The connection between the two could not be seen, but the fact that they were connected is beyond question. A glance at the part from *a* to *c* and a comparison with the nuclear portion show that the two are almost of equal length and similar in diameter. So we have here in the same figure both nucleus and its cytoplasmic envelope and both perfectly recognizable.

Figures 17 and 18 are two sections of another egg in the process of fertilization. In this egg the depression lay in the side of the cell in the section below the one shown in figure 17. Figure 18a is intended to show the nucleus of this egg viewed from the mouth of the archegonium, leaving out entirely the details of form. The nucleus is very large, quite irregular in outline, with a marked depression in the side, turned toward the mouth of the archegonium. The colorless coils *c*, *c'*, of the spermatozoid lie in this depression just below the plane of the section in figure 17, viewed from above in figure 18a. The point *d*, figure 18a, shows the end of the nuclear portion of the spermatozoid just inside the nucleus of the egg still connecting with the colorless portion outside the nucleus. The point of entrance lies behind the nucleolus *e* in figure 17. Once inside, the entire sperm-nucleus may be followed through the two sections. It was reconstructed in wire and drawn to scale in figure 18a. Careful examination of the end *d* in figure 17, which is continued in the portion *f* of figure 18, shows that in this case the slender pointed end was the last to enter the nucleus of the egg. The same fact is indicated in figures 10, 11 and 13, but is best demonstrated in this figure where both blunt and pointed ends may be found in the same section. This spermatozoid seems therefore to have coiled itself in the depression in the surface of the cell. It then broke through the cytoplasm into the egg

nucleus and slipped out of its cytoplasmic envelope, large end first, directly into the nucleus. The colorless coils *c*, *c*,' are the cytoplasmic envelope and lie entirely outside the nucleus. The pointed end *d* of the sperm-nucleus has just escaped from the cytoplasmic envelope. The fact that in this case at least the entrance of the spermatozoid is effected large end first is another evidence that the attraction which leads to entrance is exerted between the nuclei. There certainly is not a complete differentiation into an anterior and a posterior end at this stage. These observations confirm the statement that the prominent spermatozoids in figure 2 are entering large end first — that is, that the sperm-*nucleus* responds directly to the stimulus which draws it into the egg.

After the sperm-nucleus has entered the egg-nucleus, the fertilized nucleus shows a distinct tendency to develop a smooth contour. The irregularity in the shape of the nucleus begins to disappear. The changes which take place in the sperm-nucleus are exceedingly difficult to represent in detail. Connection is made almost immediately with the nuclear network of the egg. The surface of the sperm-nucleus begins to appear granular as if the whole chromatin surface were breaking up again into the separate granules of which it is composed. This process does not follow in reverse order the process of development, but the granules when first seen are very fine and small like those of the egg chromatin.

Figures 14, 15 and 16 are from successive sections of another egg-cell where the spermatozoid has already entered the nucleus leaving its cytoplasmic covering, *c*, figure 14, outside the nucleus. These coils lie below the plane of the drawing in figure 14 in the depression of the nuclear surface which is shown so strikingly at *d*, figure 15. In figure 14 this funnel-like depression lies below the plane of the figure and is overlaid by the nucleus. Its funnel-like character is shown in figure 15, where it comes more nearly to a point than in any other case observed. A comparison with figure 14 shows, however, that it lies in the side of the nucleus and opens on one whole side instead of only at the mouth of the funnel. The sperm-nucleus here shows considerable progress in the process of disintegration. Numerous strands of

nuclear network are seen leading out from it bearing chromatin granules. The surface of the spiral itself has begun to show granule-bearing strands disposed over a groundwork which in some places appears to be homogeneous, in others shows traces of a fibrillar structure. In the neck of the archegonium, as shown in figure 16, a portion of the spermatozoid appears broken into short segments. I have seen many spermatozoids broken in this way. The segments formed in the various cases when compared suggest that they represent structural differences resulting in a characteristic mode of disintegration. The same character appears in figure 22 where the breaking of the segments has taken place after the entrance of the sperm-nucleus into the egg nucleus. I have not, however, succeeded in finding any structural basis for such segmentation.

Figures 19 and 20 show two sections of another egg in which fertilization has already taken place. Here again (fig. 19) we find the cytoplasmic envelope in the depression in the side of the nucleus. The extent of this depression may be seen by comparing the vertical diameter of the nucleus in figure 19 with the same diameter in figure 20. Although this cytoplasmic part is fainter and less distinct here than in figure 13, it can be followed easily and seems to be attached to the nuclear membrane of the egg at *a*. Only about a coil and a half of the sperm-nucleus can be seen here, but it still retains clearly its spiral character. Figures 23, 24 and 25 are successive sections of another egg. In figure 25 the cytoplasmic remnant can be seen in the cytoplasm outside the nucleus. In figure 24 two pieces of nuclear material are seen, one of which closely envelops a large nucleolus. The same occurs in figures 18 and 19, where the nucleolus *n* is inclosed by the turn formed by the union of *a*, figure 18, and *b*, figure 17. In figure 23 a large coil of the sperm-nucleus lies in the plane of the section. The disorganization of the spermatozoid has become quite marked here, especially in the portion *c* where it is reduced to a mass of granules showing few of the characters of the spiral nucleus as it enters.

As a matter of precaution against mistakes the eggs shown in figures 13, 17 and 18, and 23, 24 and 25, have been recon-

structed in wax and gelatine. The spermatozoids have been carefully traced out their entire length and wire models of them have been made with the same magnification as the drawings, so that their course could be accurately determined (fig. 13).

If we take figure 26 as a still later stage in the fusion of the chromatin masses of the fertilized egg, we see that the changes are not the exact reverse of spermatogenesis. The destructive changes in the body of the spermatozoid are not so regular as the constructive changes. Instead of gradually swelling to show the strands, then short bars and finally the large distinct granules of chromatin so prominent in development, it now appears fringed on all sides with fine fibers or threads. These fibers bear very fine granules of chromatin like those of the egg nucleus, finer than the granules of the ordinary resting nucleus. Its surface appears broken or roughened by this process. These fibers are so abundant and so grouped and massed together in places as to form tangled masses like tufts of hair or cotton where the individual fiber is immediately lost to view. The entire surface is very soon involved in this disintegration process. In this we get further evidence that the nuclear portion of the spermatozoid is not truly homogeneous, if the study of its origin and cross-section are not sufficient. A large part of the mass appears fibrous. Probably these threads are the same material as the nuclear network — achromatin or linin. The chromatin granules as they separate from the mass can be seen along these threads. Sometimes the central mass shows the same tendency to stain red with safranin which was so noticeable in spermatogenesis. An identification of the fibrous material, which is linin or kinoplasm, with the red-staining central mass, which is probably fibrous also (and this as the derivative of the large nucleoli of the spermatid), while not demonstrable in the material studied, offers the most satisfactory explanation of the successive appearances of these substances. In the spermatid the two or more large nucleoli disappear when spermatogenesis begins and a red-staining "ground substance" appears which can be traced in the adult spermatozoid. Inside the egg nucleus we see the red substance at times, but always see the

fibrous material which seems to come directly from the central mass of the spermatozoid. While the evidence is not conclusive, the presence of a comparatively large amount of kinoplasmic material in the ordinary nucleus is good ground for expecting to find it here in some form. The active movement of the nuclear portion while the ripe spermatozoid swims through the water would call for the presence of such material. Its ability to move itself while entering the egg nucleus without the assistance of cilia or cytoplasmic materials points toward the same conclusion. Further, as noticed before, if we compare the chromatin matter as found in the egg in such figures as we have here, with the whole mass of the spermatozoid nucleus regarded as "homogeneous chromatin," the spermatozoid would carry in with it a great preponderance of chromatin. There certainly is no such extrusion of non-chromatin in material from the sperm-nucleus as Ikeno has figured for *Cycas revoluta*. Whether Ikeno is correct or not, in claiming that only chromatin is essential in fertilization, we certainly have here much other material carried into the egg-nucleus. The entire nuclear portion of the spermatozoid enters bodily into the nucleus of the egg without (here my observations agree with Ikeno for *Cycas*) increasing the size of the egg nucleus to any appreciable extent. The resulting segmentation nucleus shows no increase in size, but does present a doubling of the quantity of its chromatin. Only that portion of the spermatozoid which comes to lie inside of the nucleus of the egg is known to have any function in fertilization, in forms which like the ferns have *no centrosome*. Whatever its value may be, the cytoplasmic portion of the spermatozoid is absorbed in the cytoplasm of the egg and its effects cannot be traced.

The segmentation nucleus gradually loses its irregular contour and begins to assume an elliptical outline (fig. 26). The depressions in the surface of the cell and nucleus disappear. The cytoplasm usually shows a more markedly radiate arrangement outward from the nucleus. In the same figure (fig. 26) I find in the cytoplasm fibers running from the nuclear membrane toward the periphery of the cell which correspond in course and staining capacity with the fibers of

the spindle. This figure may then be regarded as representing the earliest indications of the first division spindle in the embryo and the process of fertilization must be regarded as completed.

Although in the course of this investigation I have sectioned many embryos, I have not yet attempted to carry my investigation further into the details of the formation of the embryo itself.

The observations above and a consideration of my figures give convincing evidence that the course of fertilization in *Aspidium* and *Adiantum* at least, and probably in many other ferns, differs greatly from the generally accepted description. The entrance of an unchanged sperm-nucleus into the egg nucleus has been described already by Shaw for *Onoclea* and Ikeno for *Cycas*. Shaw has found the spermatozoid entering the egg-cytoplasm. He has figured the sperm-nucleus within the egg-nucleus. I have been able to show the unchanged spermatozoid as it lies coiled in the depression in the surface of the cell and to trace it into the cytoplasm of the egg, through the nuclear membrane, to the actual mixing of the chromatin of spermatozoid and egg. I have shown that the entire sperm-nucleus slips out of the cytoplasmic envelope, or mantle (to use Ikeno's term), just as it reaches the nuclear membrane of the egg, so that it passes directly from its own cytoplasmic sheath into the egg nucleus. In *Cycas* it appears to leave its cytoplasm near the periphery of the egg. The nucleus of the spermatozoid carries in with it other material besides chromatin. This material forms the central mass, and is surrounded by the chromatin. Such a conclusion agrees with the ordinarily accepted views of fertilization more closely than with the conclusions of Ikeno. The whole nuclear portion of the spermatozoid here enters the nucleus directly. In the fertilization figures for animals such as *Nereis* (Wilson, p. 141) we find that the sperm-nucleus has swollen and assumed all the characters of an ordinary nucleus, so that the gametes are equal, not only qualitatively but quantitatively, at the time of fusion. In the phanerogams, as far as described, we never find these changes in structure in the generative nucleus of the pollen-tube which

are characteristic of spermatogenesis. Fusion is there a union of entire nuclei. The generative nucleus is carried in the end of the pollen-tube until in contact with the egg or nearly so. Similarly in the fern the entire nucleus is carried in its cytoplasmic envelope until it is actually within the cytoplasm of the egg cell.

Summary of Results.

1. As the spermatozoid enters the mouth of the archegonium it consists of a proximal (with reference to the egg) cytoplasmic portion of about one and a half coils and a distal nuclear portion of about two spiral coils.

2. The cytoplasmic forward end contains or is partially derived from the so-called "blepharoplast" and bears numerous long cilia. This part either becomes disconnected entirely before the spermatozoid reaches the egg, or, becoming functionless, is turned backward and dragged passively along into the cytoplasm of the egg.

3. The sperm-nucleus consists of an outer homogeneous appearing chromatin mass and an inner "ground substance" derived apparently from the nucleoli of the spermatid. The whole body is surrounded by a cytoplasmic envelope.

4. The nucleus once inside the archegonium displays the power of motion toward and into the egg independently of its ciliate anterior end, which indicates that the attractive impulse which leads to fertilization is exerted directly between the nuclei.

5. The egg has a concave depression in its outer surface in which the spermatozoid is found coiled before fertilization.

6. The egg nucleus has an irregular branching form extending through the cytoplasm in all directions. It contains one or more prominent nucleoli and a network bearing extremely minute chromatin granules. It has on its outer surface a concavity similar to and nearly parallel with the concavity in the surface of the cell, and approaches most nearly to the surface at this point.

7. The nuclear coils of the spermatozoid lying in the concavity of the egg break their way into the cytoplasm of the

egg toward the nucleus at the point where it lies nearest the surface. The nucleus itself is still inclosed in the cytoplasmic envelope. When it reaches the nuclear membrane it finds or breaks an opening into the egg nucleus, and, escaping from its cytoplasmic covering, passes unchanged into the nucleus of the egg. The cytoplasmic envelope is left in the egg cytoplasm where it disappears.

8. The entrance of the sperm nucleus here, as in *Cycas*, does not increase the size of the egg nucleus.

9. After entering the egg nucleus the sperm nucleus begins at once to assume the "resting stage" by breaking up into the nuclear network or reticulum bearing fine chromatin granules. This network is from the first so fused or entangled with the network of the egg nucleus already present that it is impossible to distinguish any difference between the sexual elements in the fertilized egg after the form of the spermatozoid disappears.

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EXPLANATION OF ILLUSTRATIONS.

Plates XXXVI.–XXXVIII.

Figures 1 to 41, except figures 6 and 26, are magnified 850 diameters. Figures 6 and 26 are magnified 1100 diameters, and figure 42 is magnified 2550 diameters. Figures 43 to 49 are diagrams drawn with a camera.

1, Vertical longitudinal section of archegonium showing egg-cell and spermatozoids coiled upon it. Other spermatozoids are seen entering the

neck of the archegonium. — 2–3, Successive sections of an archegonium. The spermatozoids *a* and *b* are seen entering blunt end first. In Fig. 3 a spermatozoid is seen in the depression in the surface of the egg-cell. — 4–5, Sections of another archegonium. The spermatozoid *f* is seen entering the egg-cytoplasm; *s* is a spermatozoid in the neck of the archegonium. *c* is the cytoplasmic anterior end. *c'* same for *f* turned backward. — 4a, A spermatozoid which lay in the mouth of an archegonium, *c* its cytoplasmic anterior end already disconnected. — 6–8, Successive sections of an egg; *s*, spermatozoid entering. — 9, Vertical longitudinal section of archegonium with a spermatozoid lying in the neck and another entering the egg. — 10, Fertilization of the egg. *v*, Depression in surface of egg appearing as a hole in section with the spermatozoid *f* partially coiled in it. — 11, Fertilization of the egg; spermatozoid entering as before. — 12, Fertilization of the egg; spermatozoid entering the egg at its tip. — 13–13a, Entrance of sperm-nucleus into egg-nucleus. *a–b* cytoplasmic envelope of nucleus lying outside egg-nucleus and extending into it at *b*. Fig. 13a is a diagram of Fig. 13, turned 90 degrees on the axis *x–y*. — 14–16, Fertilized egg in successive sections. *d*, a conical depression in surface of nucleus; *c*, cytoplasmic anterior end left outside the egg-nucleus. — 17, 18, and 18a, two successive sections of a fertilized egg and a diagram of the same egg viewed from above; *c*, *c'* the cytoplasmic envelope of the sperm nucleus left outside the egg nucleus. — 19–20, Two sections of another fertilized egg, showing the cytoplasmic sheath outside the nucleus in Fig. 19 and the sperm-nucleus inside the egg-nucleus in Fig. 20. — 21–22, Fertilized eggs showing unchanged sperm-nuclei. — 23–25, Successive sections of a fertilized egg. — 26, Fertilized egg where the disorganization of the sperm-nucleus has gone much farther. — 27–41, Stages in the development of the spermatozoid. — 42, Cross-section of ripe spermatozoid. — 43, Diagram of archegonium in vertical longitudinal section. — 44–48, Diagrams of the egg-cell. — 49, Cross-section of prothallium showing two embryos in the same calyptra; *c*, cotyledon; *F*, foot; *r*, root; *s*, stem.

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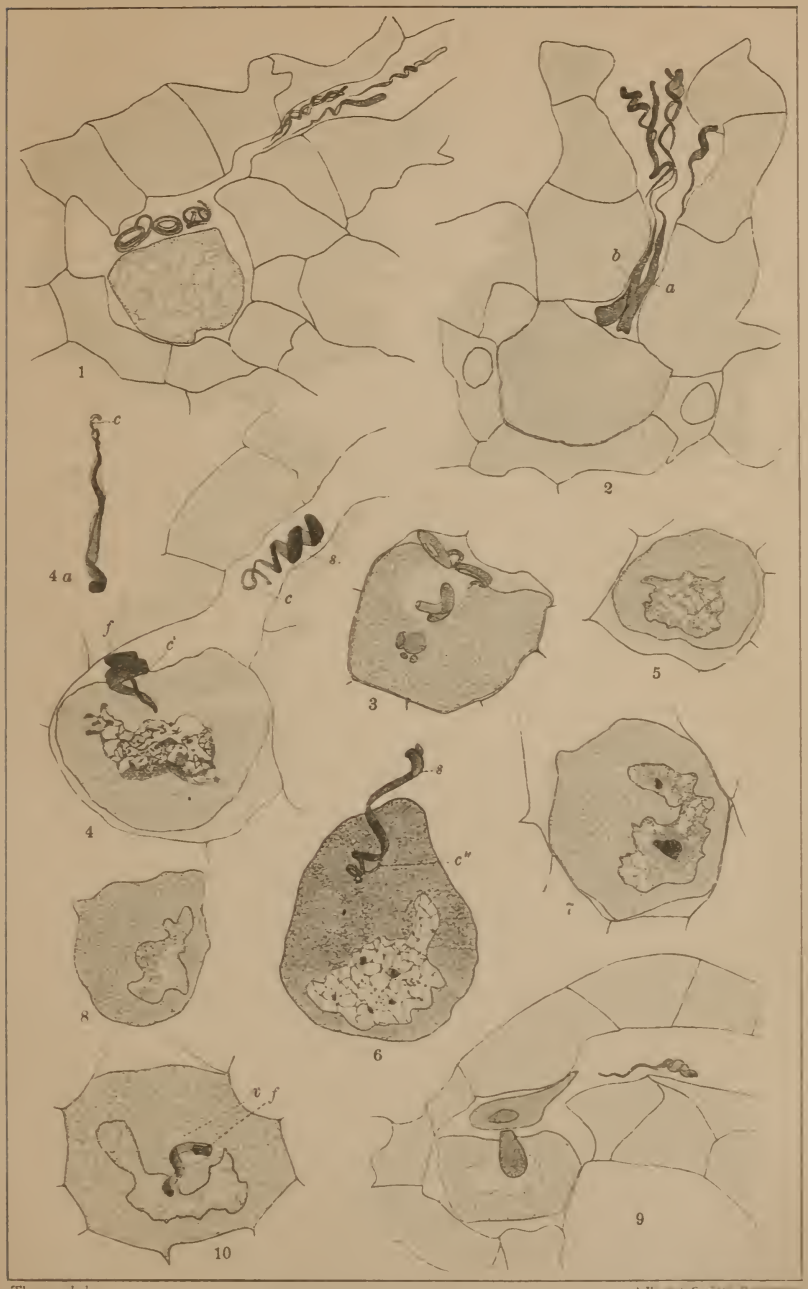

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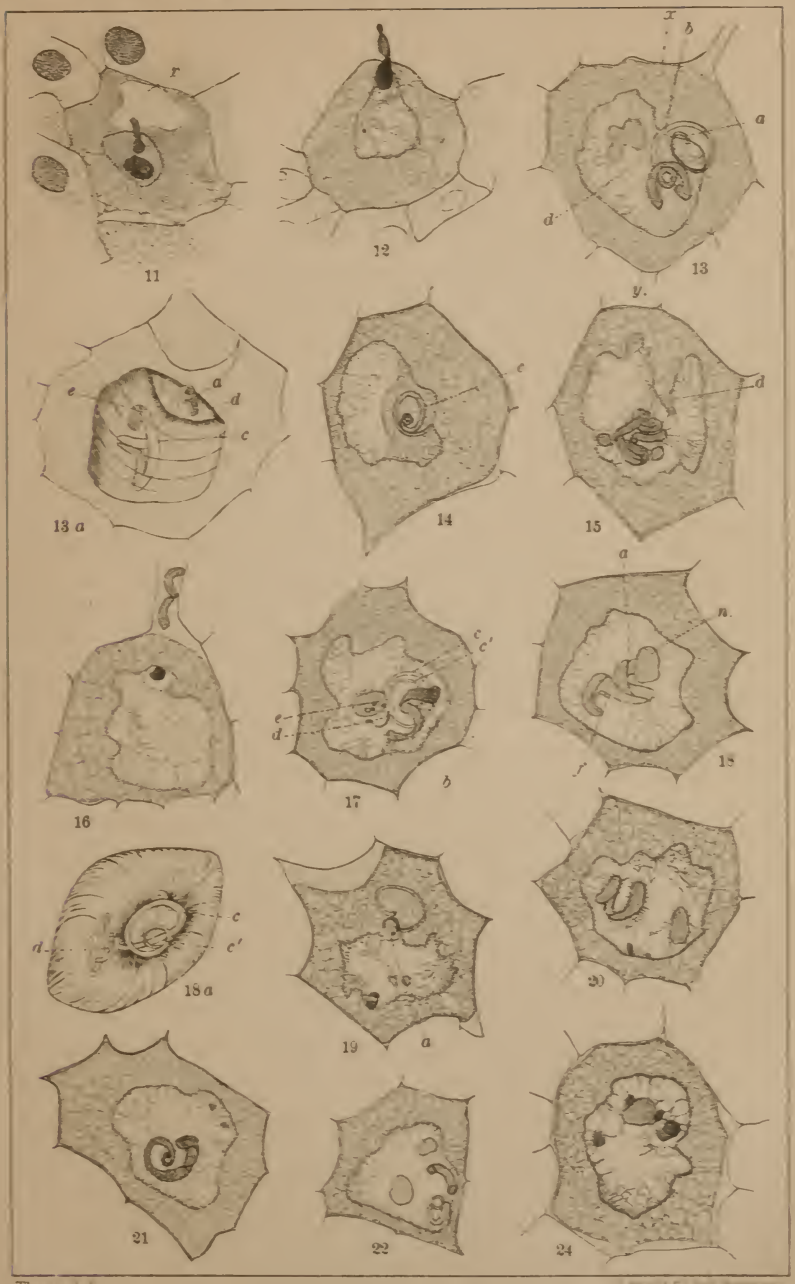
FERTILIZATION IN FERNS.



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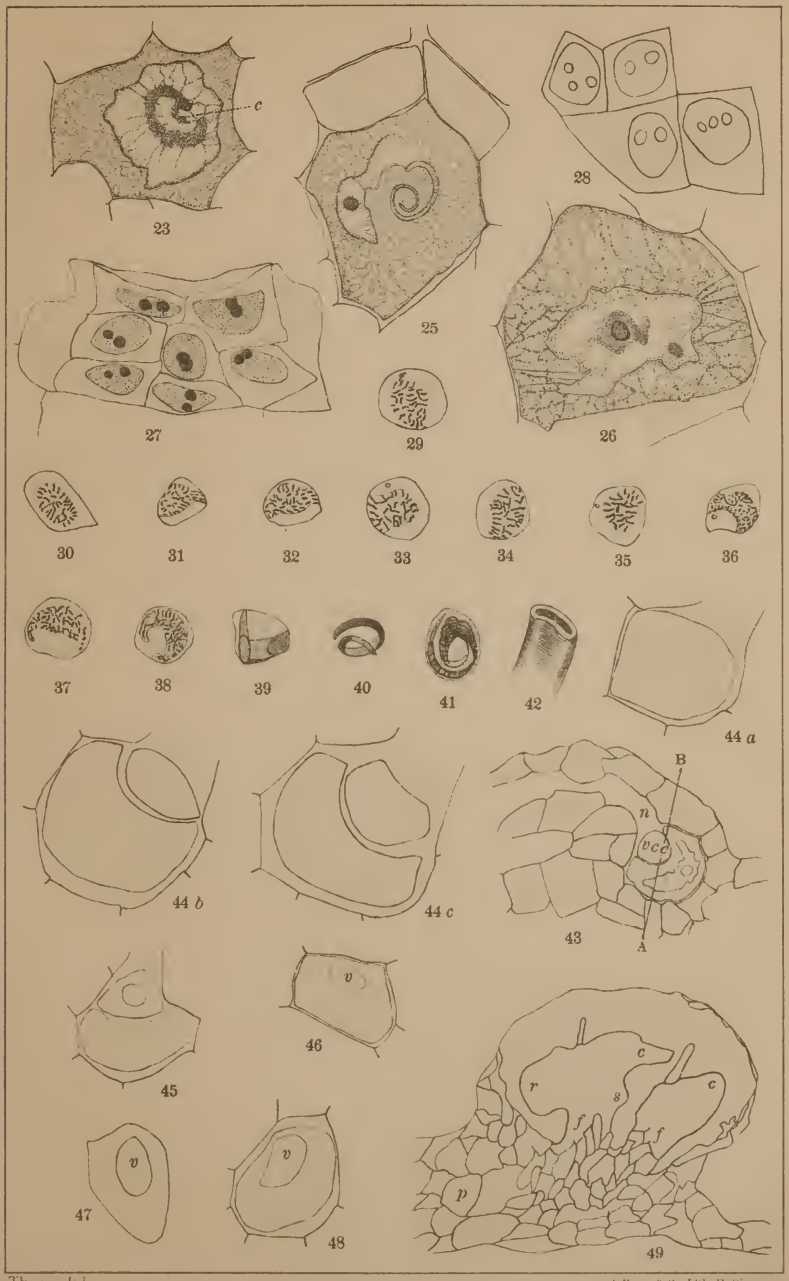

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FERTILIZATION IN FERNS.